REMARKS

Applicants have received and reviewed an Office Action in the nature of a Restriction Requirement and a species election requirement dated September 17, 1996. In response, Applicants select the claims of Group I, with traverse. In addition, Applicants present new claims 17-21 to be added to Group I. No new matter is added in the newly presented claims. In response to the species election requirement, Applicants elect species III, with traverse. Applicants reserve the right to pursue any subject matter removed from consideration in this application in one or more continuation or divisional applications.

Applicants respectfully traverse the requirement to elect a single group of claims and the requirement to elect a single species. The reasons for this traverse are described in the remarks below. The Examiner is respectfully requested to consider the remarks, withdraw the requirement to elect a group of claims and species, and to examine all of the claims.

Restriction Requirement

The Examiner asserts that the application contains several inventions or groups of inventions that are not so linked as to form a single inventive concept under PCT Rule 13.1. First, Applicants respectfully bring to the Examiner's attention the written opinion issued by the International Preliminary Examining Authority dated June 10, 1994 and the International

Preliminary Examination Report dated December 6, 1994. These reports from the International Preliminary Examination Authority found that there was unity of invention and a common inventive step throughout Applicants' invention. Applicants respectfully note that although the conclusion of the International Preliminary Examination Authority is not binding on the Examiner, it is evidence that Applicants' claims are characterized by a single general inventive concept.

The Examiner asserts that the inventions listed as Group I-III do not relate to a single inventive concept because the paramagnetic particles or beads used in Applicants' invention are not considered to be a special technical feature. The Examiner contends that such beads and their use are well known in the art, and as obvious over Winter et al. (EP 016,552) in view of Connelly et al. (United States Patent No. 5,422,277) and Forrest et al. (United States Patent No. 4,659,678). In fact, the references cited by the Examiner do not make obvious Applicants' invention, as is described below. It is believed that there is a single inventive concept throughout Applicants' invention.

Applicants' Invention

Applicants' claimed invention includes a method in which monoclonal antibodies bound to magnetic beads can be used for specific selection and detection of subpopulations of cells in a heterogeneous cell population. Usually, the target cells

are a very minor fraction of the total cells present in the suspension. Applicants' method permits selection of the target cells with a very high degree of purity compared to methods previously known. Moreover, the size of the beads used in Applicants' procedure is important for the detection part of the invention, as the beads bound to the target cells can be used for identification in a microscope.

In Applicant's method, the binding of the antibody-coated beads must be highly, for example close to 100%, specific. This requisite high specificity is achieved with the procedure described in the invention, but not in the prior art methods, and is believed to be related to the specificity of the antibodies, to a uniform distribution of bound antibodies on the surface of the beads and to the use of superparamagnetic beads with homogenous distribution of magnetism within the beads. Prior art methods do not give such a high specific binding of antibody-coated microspheres. In Applicants' method, a factor for obtaining the specificity is that the cells are kept at 4°C during the procedure until microscopy.

The Cited Prior Art

The Examiner refers to methods described by Widder et al. (EP 106,552), Connelly et al. (United States Patent No. 5,422,277), and Forrest et al. (United States Patent No. 4,659,678).

The procedure described by Widder et al. includes protein A associated with microspheres and further reacted with selected antibodies. The microspheres used consist of polymer matrix material such as the protein albumin, the magnetic particles are Fe_3O_4 prepared together with protein A, which in part is present on the exterior surfaces of the microspheres.

This is distinct from Applicant's invention in several ways. First, the type of polymers and the use of protein A result in considerable nonspecific binding/adhesion to non-target cells of any type. Second, polyclonal antibodies as used by Widder et al. can also increase nonspecific binding to non-target cells. Third, the particles will have a wide range of sizes, amplifying this problem. Fourth, the magnetic particles in the microspheres give very low strength of magnetic separation.

A feature of Applicants' invention is that it becomes possible to select and detect target cells that represent a minor fraction of the total number of cells in a heterogeneous cell suspension. The method described by Widder et al. is ineffective for this purpose. Moreover, the Widder et al. reference does not provide any knowledge that teaches or suggests Applicants' solution of these problems.

As described in claim 1.1, we teach the use of paramagnetic or superparamagnetic particles with very high magnetic attraction capacity. These can be coated either directly with antibodies directed against antigens expressed on target cells or with anti-mouse or anti-human antibodies capable

of binding to the Fc-portions of the said antibodies. This means that it is either a direct or a double layer antibody coating of the particles. In claim 1.2.2, we describe the incubation of the coated beads with the cell suspension preferably for 30 minutes at 4°C under gentle rotation. As mentioned above, performing the method at 4°C, unexpectedly, has been found to dramatically decrease any nonspecific adhesion of the particles to non-target cells. In claim 1.4.1, mild detergents are described as another means to reduce nonspecific binding. Detergents are, however, not always necessary.

Connelly et al. teach the use of fixatives which is not warranted or required for the purposes of the present application. Use of fixatives kills the cells. In contrast, in Applicants' claimed method, for many purposes it is important to keep the cells alive. In our claim 1.4.2 use of fixatives are only of importance for some purposes in which visualization of intracellular molecules might be important.

Forrest et al. teach a sandwich assay using fluorimetric or enzyme labeling of the antibodies for subsequent identification. Here the Examiner refers to the use of avidin/biotin. In claim 1.4.3, this is also mentioned, but only as one of several means of visualizing additional cell markers, and not for use as one of the steps necessary to bind the magnetic particles to the target cells. Moreover, Forrest et al. did not teach an assay to detect cells, but only soluble antigens, i.e., very small molecules compared to whole cells.

The references cited by the Examiner either alone or in combination, fail to teach or suggest Applicants' claimed invention. Accordingly, there is a single inventive concept throughout the claims.

The Species Election Requirement

The Examiner asserts that the application contains claims directed to more than one species of the generic invention. The Examiner contends that the species are not so linked as to form a single inventive concept. As described above, Applicants' claims include a single inventive concept relating to using monoclonal antibodies bound to magnetic beads for specific selection and detection of subpopulations of cells in a heterogeneous cell population. Applicants respectfully remind the Examiner that such unity of invention and inventive concept was found by the examiner at the International Preliminary Examination Authority. Such unity of invention is believed to make unnecessary species election.

Applicants have elected Species III, which the Examiner describes as abnormal cells expressing integrins, or adhesion membrane molecules, or MDR proteins or growth factor receptors or their oncogenic products. The Examiner asserts that Species III is included in claims 6, 10-12, and 13, while claims 1, 7-9, and 16 are generic. In the event that a generic claim is not found allowable, Applicants elect the species of Group III, including abnormal cells as described in claims 6, 10-12, and 13. The

newly presented claims 17-21 are also generic. The abnormal cells of the invention include cells with abnormal development patterns, such as primary metastatic cancer cells, and abnormal cells associated with non-neoplastic diseases, such as cardiovascular, neurological, pulmonary, autoimmune, gastrointestinal, genitourinary, reticuloendothelial, and other disorders.

Subject to traverse, as described above, in response to the Examiner's assertion that Applicants must elect a single antigen belonging to each of the groups of adhesion molecules, or integrin, or growth receptors, etc., Applicants elect, in the event no generic claim is found allowable, the following antigens from the classes defined by the Examiner. Among integrins, Applicants select the following antigens: Vitronectin receptor ($\alpha v\beta 3$ integrin), ICAM-I (CD54), P-selectin/GMP-140, CD44-variants, N-CAM(CD56), and E-cadherin. Among growth factors, Applicants select the following: Laminin receptor, GD_2, EGF receptor, and TNF-receptor. Among carcinoma antigens, Applicants select the following carcinoma antigens: Le^Y, CEA, High molecular weight antigen (HMW 250.000), TP-1 and TP-3 epitope, MOC-31 epitope (cluster 2 epithelial antigen), MUC-1 antigens (such as DF3-epitope (gp290kD)), Polymorphic epithelial mucins, Prostate specific membrane antigen (Cty-356), Ovarian carcinoma OC125 epitope (mw 750 kD), Pancreatic HMW glycoprotein, TAG 72, Bladder carcinoma antigen, Hepatocellular carcinoma antigen Mw900kD, M.48kD colorectal carcinoma antigen, Lung carcinoma

antigen Mw350-420kD, UJ13A epitope, Mel-14 epitope, Mw18-22kD antigen, β-microglobulin, Apo-1 epitope, and pan-human cell antigen.

It is believed that each of the above listed antigens is sufficiently closely related as to define a single species.

Summary

Applicants have selected examination of claims of Group I, and elected Species III, and traversed both the restriction and species election requirements. Applicants respectfully request examination of all of the claims as filed and the newly presented claims.

The Examiner is invited to telephone Applicants' representative at the telephone number below if the Examiner believes that prosecution can be advanced thereby.

Respectfully submitted,

Fodstad et al.,

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